JOURNAL OF BACTERIOLOGY, Sept. 1992, p. 5489–5495
0021-9193/92/175489-07$02.00/0

Vol. 174, No. 17

MINIREVIEW

Methane from Acetate

JAMES G. FERRY

Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0305

INTRODUCTION

Anaerobic microorganisms play a major role in the global carbon cycle by remineralizing the large quantities of organic matter which enter anoxic marine and freshwater environments. Methane and CO_2 are the end products of anaerobic decomposition, which occurs in a diversity of habitats, such as the rumens of ruminant animals, the lower intestinal tracts of humans, sewage digesters, landfills, rice paddies, and the sediments of freshwater lakes and rivers. Most of the methane released is oxidized to CO_2 by the strictly aerobic methanotrophs; however, a significant proportion escapes into the upper atmosphere where methane has a major role in the greenhouse effect.

The methanogenic decomposition of organic matter requires microbial consortia composed of at least three interacting metabolic groups of anaerobes. The fermentative bacteria degrade polymers to H_2, CO_2, formate, acetate, and higher volatile carboxylic acids. The acetogenic bacteria then oxidize the higher acids to acetate and either H_2 or formate. The strictly anaerobic methane-producing microorganisms are the final group in the consortia and utilize H_2, formate, and acetate as substrates for growth. Methane producers represent the largest and most diverse group within the *Archaea* domain (57). The reader is referred to reviews that describe general aspects of the methanogenic members of the *Archaea* domain (32, 56).

About two-thirds of the methane produced in nature originates from the methyl group of acetate, and about one-third originates from the reduction of CO_2 with electrons derived from the oxidation of H_2 or formate. Two independent pathways exist for the conversion of acetate and reduction of CO_2. The pathway of CO_2 reduction to methane has been studied extensively over the past 20 years and is the subject of several reviews (9, 34, 51, 55); however, a fundamental understanding of methanogenesis from acetate has emerged only recently.

Most acetate-utilizing anaerobes from the *Bacteria* domain cleave acetyl coenzyme A (acetyl-CoA) and oxidize the methyl and carbonyl groups completely to CO_2, reducing a variety of electron acceptors (52). In contrast, the methanogenic members of the *Archaea* domain carry out a fermentation of acetate in which the molecule is cleaved and the methyl group is reduced to methane with electrons derived from oxidation of the carbonyl group to CO_2 (reaction 1).

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \quad (\Delta G^{\circ\prime} = -36 \text{ kJ/mol [55]}) \qquad (1)$$

The extreme phylogenetic difference between the *Archaea* and *Bacteria* domains raises questions regarding the comparative biochemical mechanisms of acetate activation and cleavage. The methanogenic fermentation of acetate also raises interesting questions regarding nature's solutions to the problems of methyl transfer, electron transport, and energy conservation that are unique to acetate-utilizing methanogenic microorganisms. Figure 1 depicts general features of the pathway in *Methanosarcina thermophila* as it is currently understood. The utilization of acetate as a substrate for growth and methanogenesis is confined to species within the genera *Methanosarcina* and *Methanothrix*. The pathway shown in Fig. 1, with only minor modifications, is also utilized by *Methanothrix* spp.

ACTIVATION OF ACETATE TO ACETYL-CoA

Mechanistic constraints require that acetate is first activated prior to cleavage. *Methanosarcina* spp. activate acetate to acetyl-CoA (12, 17) in a reaction catalyzed by acetate kinase (reaction 2) and phosphotransacetylase (reaction 3).

$$CH_3COO^- + ATP \rightarrow CH_3CO_2PO_3^{2-} + ADP \qquad (2)$$

$$CH_3CO_2PO_3^{2-} + CoA \rightarrow CH_3COSCoA + P_i \qquad (3)$$

$$CH_3COO^- + CoA + ATP \rightarrow CH_3COSCoA + AMP + PP_i \qquad (4)$$

These enzymes are absent in acetate-grown *Methanothrix soehngenii*, but this organism contains high levels of acetyl-CoA synthetase (28), which catalyzes the activation to acetyl-CoA (reaction 4). In each organism, one high-energy phosphate bond is expended for each acetate metabolized and represents a considerable investment considering the small amount of energy available for ATP synthesis (reaction 1).

Although *M. thermophila* is a strict anaerobe, acetate kinase from *M. thermophila* is stable in air and the enzyme is purified from the soluble fraction as an α_2 homodimer with a subunit M_r of 53,000 (3). The K_m for ATP is 2.8 mM, and the K_m for acetate is 22 mM, which reflects the relatively high K_s for acetate uptake by *Methanosarcina* spp. (30). Activity of the purified enzyme with the nucleotide triphosphates TTP, ITP, UTP, and GTP is greater than 80% of the activity with ATP. The monomeric (M_r = 42,000) phosphotransacetylase, which is stable in air, is purified from the soluble fraction of *M. thermophila* (44). The activity of this enzyme is stimulated over sevenfold by the presence of 50 mM potassium or ammonium ions; however, phosphate, arsenate, and sulfate are inhibitory. The K_m for CoA is 91 μM, and the K_m for acetyl-PO_3^{2-} is 165 μM.

The acetyl-CoA synthetase from *M. soehngenii* is also stable in air and is isolated from the soluble fraction as an α_2 homodimer with a subunit molecular mass of 73,000 Da (28). The K_m for acetate is 0.86 mM, which is consistent with the high affinity of this organism for acetate (30). The gene encoding acetyl-CoA synthetase has been cloned and sequenced; on the basis of the deduced amino acid sequence,

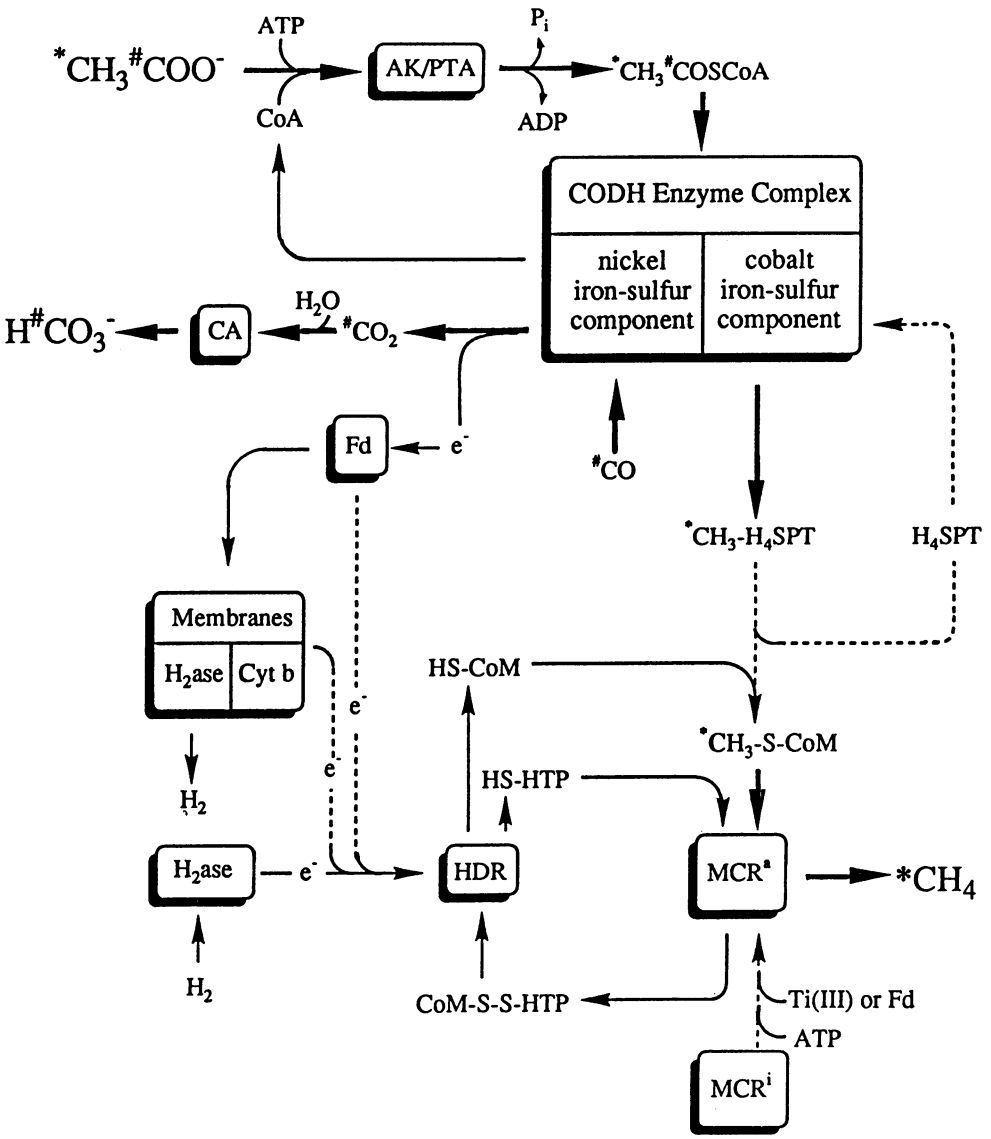

FIG. 1. Proposed pathway for the conversion of acetate to CO_2 and CH_4 in *M. thermophila* modified from Jablonski and Ferry (23). AK, acetate kinase; PTA, phosphotransacetylase; CA, carbonic anhydrase; MCR^i, inactive methylreductase; MCR^a, active methylreductase; HDR, heterodisulfide (CoM-S-S-HTP) reductase; Fd, ferredoxin; Cyt b, cytochrome *b*; H_2ase, hydrogenase; e^-, electron. Dashed lines represent gaps in understanding of the pathway. The carbon atoms are marked with * and # symbols to distinguish the two sources of carbons, the methyl and carboxyl groups, respectively.

the synthetase appears to have a domain similar to those of other ATP-binding proteins (10).

CARBON-CARBON AND CARBON-SULFUR BOND CLEAVAGE OF ACETYL-CoA

The central enzyme in the pathway of methanogenesis from acetate is CO dehydrogenase (CODH), which catalyzes the cleavage of acetyl-CoA. CODHs are widespread among anaerobes and serve a variety of functions. Several anaerobes from the *Bacteria* domain oxidize acetate completely to CO_2 and reduce various electron acceptors (52); most contain CODH which cleaves acetyl-CoA, yielding the methyl and carbonyl groups which are then oxidized to CO_2. The homoacetogenic bacteria employ CODH (acetyl-CoA synthase) in the energy-yielding Wood pathway to catalyze the synthesis of acetyl-CoA. CODH is also utilized by the methanogenic members of the *Archaea* domain for cell carbon synthesis from CO_2 (38). Microorganisms from the *Archaea* and *Bacteria* domains are at the extremes of bacterial evolution (57), and comparative studies of CODHs from these widely divergent organisms are likely to enhance an understanding of the mechanism and molecular evolution of this important enzyme.

In *M. thermophila*, a COOH enzyme complex cleaves the C-C and C-S bonds of acetyl-CoA, as demonstrated by an exchange of CO with the carbonyl group of acetyl-CoA (46). The complex also oxidizes CO to CO_2 and, therefore, is referred to as a CODH enzyme complex; however, the primary function is cleavage of acetyl-CoA during growth on

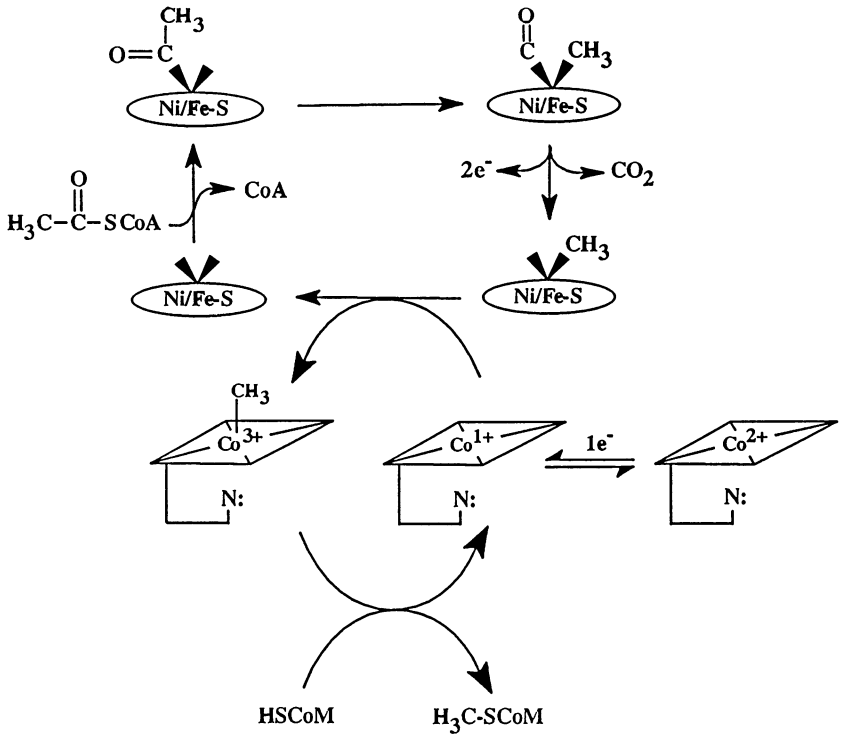

FIG. 2. Proposed mechanism for acetyl-CoA cleavage by the CODH complex from *M. thermophila*. The oval represents the Ni/Fe-S component of the complex. The rhombus represents the corrinoid ring of factor III in the Co/Fe-S component of the complex, and N: represents the nonliganding nitrogen atom of the base. e^-, electron.

acetate. After cleavage of acetyl-CoA, the enzyme-bound carbonyl group is oxidized to CO_2 and the methyl group is transferred to the cofactor tetrahydrosarcinapterin (H_4SPT) (13, 16), which is a one-carbon carrier found only in members of the *Archaea* domain. The pterin moiety of H_4SPT is similar to tetrahydrofolic acid in structure and chemistry (9).

The five-subunit *M. thermophila* CODH complex can be resolved into two enzyme components: a 200,000-Da CO-oxidizing Ni/Fe-S component which contains 89,000- and 19,000-Da subunits, and a 100,000-Da Co/Fe-S component which contains 60,000- and 58,000-Da subunits (2). The fifth subunit (71,000 Da) of the complex has not been characterized. The CO-reduced enzyme complex has a spin-coupled Ni-Fe-C center with an electron paramagnetic resonance (EPR) spectrum indistinguishable from the Ni-Fe-C center of the CO-reduced CODH (acetyl-CoA synthase) from the homoacetogenic organism *Clostridium thermoaceticum* (48). The reduced Ni-Fe center in the *C. thermoaceticum* enzyme is the proposed site for synthesis of the acetyl moiety of acetyl-CoA from CoA, CO, and a methyl group donated by a methylated corrinoid/Fe-S protein (42, 43). Therefore, it is proposed that the Ni-Fe center in the *M. thermophila* enzyme is the site for cleavage of the acetyl moiety of acetyl-CoA (2). The Co/Fe-S component from the *M. thermophila* CODH enzyme complex contains factor III (Coα-[α-(5-hydroxybenzimidazolyl)]-cobamide), the cobalt atom of which is reduced to the Co^{1+} state with electrons donated directly by the Ni/Fe-S component (2). In this redox state, Co^{1+} is a supernucleophile which can then accept a methyl group (2).

The composition and properties of components from the CODH complex of *M. thermophila* are consistent with a proposed acetyl-CoA cleavage mechanism (Fig. 2) which is analogous to a reversal of the mechanism proposed for acetyl-CoA synthesis in *C. thermoaceticum* (42, 43). In the proposed mechanism (Fig. 2), the Ni/Fe-S component cleaves the C-C and C-S bonds of acetyl-CoA at the Ni-Fe site (2). After cleavage, the methyl group is transferred to the Co^{1+} atom of the Co/Fe-S component. The *M. thermophila* enzyme complex catalyzes an exchange of CoA with acetyl-CoA at rates fivefold greater than that of *C. thermoaceticum* acetyl-CoA synthase (46). The difference in rates may reflect the acetyl-CoA cleavage function for the *M. thermophila* enzyme, as opposed to the *C. thermoaceticum* enzyme which functions in the biosynthesis of acetyl-CoA. In addition to the C-C and C-S bond cleavage activity, it is proposed that the Ni-Fe center binds the carbonyl group and oxidizes it to CO_2. This proposed function is supported by the ability of the Ni/Fe-S component to oxidize CO and reduce a ferredoxin purified from *M. thermophila* (2). The CODH complex from *M. thermophila* also catalyzes the synthesis of acetyl-CoA from CoA, CO, and CH_3I in vitro (1); however, it is unlikely that this is a physiologically significant reaction in vivo.

The component enzymes from the *M. thermophila* complex have been characterized in greater detail by EPR spectroscopy (24). The CO-oxidizing Ni/Fe-S component contains three species of Fe-S clusters with EPR properties similar to those reported for *C. thermoaceticum* acetyl-CoA synthase (40, 41). Two of the Fe-S centers in the *M. thermophila* Ni/Fe-S component have EPR spectra typical of bacterial 4Fe-4S centers; however, the third Fe-S center has an EPR spectrum which is atypical of 4Fe-4S centers. The CO-reduced Ni/Fe-S component also exhibits the spin-cou-

pled Ni-Fe-C EPR spectrum, a finding consistent with the proposed mechanism for acetyl-CoA cleavage (Fig. 2); however, it is not known which, if any, of the Fe atoms from the Fe-S centers participate in formation of the Ni-Fe center. EPR spectroscopy of the isolated Co/Fe-S component indicates a low-spin Co^{2+} (24). There is no superhyperfine splitting from the nitrogen nucleus ($I = 1/2$) of the 5-hydroxybenzimadazole base in factor III, a result which indicates the absence of a lower axial ligand to the cobalt atom (base-off configuration). Redox titration of the $Co^{2+/1+}$ couple reveals a midpoint potential of approximately −500 mV similar to that reported for the *C. thermoaceticum* corrinoid/Fe-S protein in which the corrinoid is also in the base-off configuration (19). The base-off configuration of corrinoids changes the midpoint potential of the $Co^{2+/1+}$ couple from approximately −600 mV (base-on configuration) to a less negative value, which allows reduction to the methyl-accepting Co^{1+} redox state by physiological electron donors which have midpoint potentials around −500 mV (2, 19). The Co/Fe-S component of the *M. thermophila* enzyme complex contains a center with EPR properties similar to the 4Fe-4S center present in the *C. thermoaceticum* corrinoid/Fe-S protein (24). In summary, the biochemical properties of the enzyme components from the *M. thermophila* enzyme complex strikingly resemble properties of the *C. thermoaceticum* acetyl-CoA synthase and corrinoid/Fe-S protein, a finding which further supports similar catalytic mechanisms.

The CODH characterized from *Methanosarcina barkeri* is purified as an $\alpha_2\beta_2$ oligomer with subunit M_rs of approximately 90,000 and 19,000, similar to the CO-oxidizing Ni/Fe-S component of the *M. thermophila* complex (18, 36). Recently, it was reported that the *M. barkeri* $\alpha_2\beta_2$ CODH can be purified in an enzyme complex which also contains a corrinoid protein (16). The complex catalyzes cleavage of acetyl-CoA and transfer of the methyl group to H_4SPT (16); however, the properties and function of the corrinoid protein were not investigated. The *M. barkeri* $\alpha_2\beta_2$ CODH component contains Ni and Fe; however, no EPR signals attributable to Ni have been reported. Six 4Fe-4S clusters per $\alpha_2\beta_2$ tetramer are indicated by core extrusion experiments (36). Low-temperature EPR spectroscopy has identified a 4Fe-4S cluster with a midpoint potential of −390 mV. A second low-temperature EPR signal is obtained from the reduced enzyme which is atypical of bacterial 4Fe-4S centers; the spectrum is perturbed on incubation with CO. This atypical Fe-S spectrum is observed in whole cells of *M. barkeri* during methanogenesis, a result which indicates that the cleavage of acetate yields a moiety that CODH recognizes as CO (37).

Unlike the *Methanosarcina* enzymes, the CODH from *M. soehngenii* can be purified in the presence of air but requires strictly anaerobic conditions for CO-oxidizing activity (29). The native enzyme is an $\alpha_2\beta_2$ oligomer composed of subunits with molecular masses of 79,400 and 19,400 Da and contains Ni and Fe. Thus, the *M. soehngenii* enzyme is similar to the *Methanosarcina* enzymes, except that it has not been purified in association with a corrinoid-containing protein. The *M. soehngenii* enzyme catalyzes the exchange of CO with the carbonyl group of acetyl-CoA, demonstrating C-C and C-S cleavage activity (26). The gene encoding the α subunit has a deduced amino acid sequence which shows homology with acyl-CoA oxidases; however, no consensus sequences involved in acetyl-CoA or CoA binding are evident (11). The deduced amino acid sequence of the largest subunit also contains eight cysteine residues with spacings that could accommodate Fe-S centers; accordingly, the anaerobically purified (reduced) enzyme exhibits two low-temperature EPR signals (26, 27). One of these signals is attributable to a 4Fe-4S center, and the other is atypical of bacterial 4Fe-4S centers. The atypical signal is substoichiometric in intensity and partially disappears when the enzyme is incubated with CO. It is postulated that this signal may arise from a 6Fe-6S prismanelike center. No EPR signals attributable to a Ni center have been reported for the *M. soehngenii* enzyme.

In summary, all CODHs characterized from acetate-grown methanogenic organisms have a similar subunit composition, contain Ni, contain 4Fe-4S centers, and have a Fe-S center with unusual EPR properties. A Ni-Fe center is the proposed site for cleavage of acetyl-CoA, but structural details of the center are unknown. In *Methanosarcina* spp., the CODHs are associated with a corrinoid-containing protein which is postulated to accept the methyl group of acetyl-CoA.

METHYL TRANSFER TO HS-CoM AND REDUCTIVE DEMETHYLATION OF CH_3-S-CoM TO METHANE

After cleavage of acetyl-CoA, the methyl group is ultimately transferred to HS-CoM (2-mercaptoethanesulfonic acid) (9) involving methyl-H_4SPT as a suspected intermediate (13, 16), which implies a requirement for at least two methyltransferases. An enzyme (M_r = 34,000) with methylcobalamin:HS-CoM methyltransferase activity from acetate-grown *M. barkeri* has been described (15); however, it is unknown if methyl-H_4SPT can replace methylcobalamin. Evidence has been obtained for the involvement of a corrinoid enzyme in methanogenesis from acetate in *M. barkeri* (54) and two corrinoid-containing proteins (480,000 and 29,000 Da) from *M. barkeri* have recently been described (7). The 480,000-Da protein is methylated at the onset of methanogenesis in whole cells and demethylated when methanogenesis stops, a result which indicates an involvement in methyltransfer. The 29,000-Da protein is methylated only when reductive demethylation of CH_3-S-CoM is inhibited. Thus, several potential methyltransfer proteins have been identified but their function in the pathway is unknown.

The final step in the utilization of all methanogenic substrates is the reductive demethylation of CH_3-S-CoM to CH_4 catalyzed by CH_3-S-CoM methylreductase. The two electrons required for the reduction derive from the sulfur atoms of CH_3-S-CoM and HS-HTP (7-mercaptoheptanoylthreonine phosphate) (9), yielding the heterodisulfide CoM-S-S-HTP. In the pathway for methanogenesis from acetate, the mixed disulfide is reduced to the corresponding sulfhydryl forms of the cofactors with electrons originating from oxidation of the carbonyl group of acetyl-CoA (21, 47). The thermodynamics of both heterodisulfide formation and reduction (reactions 5 and 6) suggests a potential for electron transport coupled to ATP synthesis.

$$CH_3\text{-S-CoM} + \text{HS-HTP} \rightarrow CH_4 + \text{CoM-S-S-HTP} \quad (\Delta G^{\circ\prime} = -45 \text{ kJ/mol [51]}) \quad (5)$$

$$\text{CoM-S-S-HTP} + H_2 \rightarrow \text{HS-CoM} + \text{HS-HTP} \quad (\Delta G^{\circ\prime} = -40 \text{ kJ/mol [51]}) \quad (6)$$

All methylreductases studied thus far contain factor F_{430} which is a yellow, Ni-containing, structural hybrid of the corrin and porphyrin ring systems (9). The mechanism involving F_{430} is unknown but it is postulated that a Ni(I)-S-HTP species reacts with CH_3-S-CoM to form Ni(II)-CH_3, which returns to the nonligated Ni(I) species, yielding CoM-S-S-HTP and CH_4 (4, 25, 37, 39). The methylreductase purified from *M. thermophila* has a subunit composition of

$\alpha_1\beta_1\gamma_1$ with M_rs of 69,000, 42,000, and 33,000 (23). The native enzyme (M_r = 141,000) contains 1 mol of F_{430} and utilizes HS-HTP as the electron donor. The as-isolated enzyme requires a reductive reactivation which can be accomplished in vitro with ferredoxin purified from *M. thermophila* (49); ATP is not required but stimulates the reactivation. Ti(III) citrate can substitute for ferredoxin in a nonenzymatic reaction. It is proposed that the methylreductase is isolated in two forms: a ready form that can be reactivated with reduced ferredoxin and an unready form unable to be reductively reactivated unless converted to the ready form by an unknown mechanism that requires ATP (Fig. 1); however, the physiological significance of this activation is unknown. The *Methanosarcina mazei* methylreductase contains F_{430} and has a native molecular mass of 283,400 Da with subunits of 68,000, 43,215, and 30,500 Da in a $\alpha_2\beta_2\gamma_2$ configuration (53). The CH_3-S-CoM methylreductase from *M. soehngenii* has the same subunit composition and utilizes HS-HTP as the electron donor (31); a requirement for reductive activation of this enzyme was not investigated. Immunogold labelling of several acetate-grown *Methanosarcina* species and *M. soehngenii* indicates that the methylreductase of these acetotrophic organisms is primarily located in the cytoplasm (53); however, the cells were grown with abundant nickel in the growth medium, conditions which may have influenced the amount of cytoplasmic methylreductase relative to membrane-associated enzyme (5).

ELECTRON TRANSPORT AND BIOENERGETICS

A relatively small amount of energy is available from the conversion of acetate to CH_4 and CO_2 (reaction 1), and one high-energy phosphate bond is already expended in the activation of acetate. Thus, these organisms must have evolved a very efficient mechanism for energy conservation. There are no obvious reactions leading to a substrate-level phosphorylation; however, several lines of evidence support a chemiosmotic mechanism for ATP synthesis. Acetate-degrading whole cells of *M. barkeri* generate a proton motive force of −120 mV (45). It is likely that the transport of electrons from the carbonyl group of acetyl-CoA to CoM-S-S-HTP is dependent on membrane-bound carriers involved in generation of the proton motive force; indeed, the membranes of *M. thermophila* and *M. barkeri* contain cytochrome *b*, multiple Fe-S centers, and possibly rubredoxin (35, 50). In addition, over 50% of the heterodisulfide reductase activity in *M. barkeri* is associated with the membrane fraction (47). CO-dependent methylreductase activity is stimulated by the addition of membranes to the soluble fraction, a result which further supports the involvement of a membrane-bound electron transport chain (50). Although coenzyme F_{420} (a 5-deazaflavin) (9) is an important electron carrier in the CO_2-reducing pathway, it is present only in low levels in acetate-grown cells of *M. barkeri* and is not required for conversion of acetyl-CoA to methane in cell extracts of this organism (13). However, F_{420} may be involved in oxidation of the methyl group of acetate to CO_2 to provide electrons for reductive biosynthesis (see below).

Recent results strongly implicate an involvement of ferredoxin in electron transport. Ferredoxin is required for methanogenesis from acetate in extracts of *M. barkeri* (14) and is a direct electron acceptor for the Ni/Fe-S component of *M. thermophila* (2); however, the carriers mediating electron flow from ferredoxin to heterodisulfide reductase are unknown. The gene encoding the ferredoxin from *M. thermophila* has a deduced amino acid sequence which contains eight cysteines in a spacing characteristic of 2[4Fe-4S] ferredoxins from the *Bacteria* domain (8). The gene is transcribed in cells grown on either acetate, trimethylamine, or methanol, a result which suggests a requirement for the ferredoxin in the metabolism of each substrate. A ferredoxin-dependent evolution of CO_2 and H_2 from acetyl-CoA has been demonstrated in extracts of *M. barkeri* (14) and a CO-oxidizing:H_2-evolving system (reaction 7) in *M. thermophila* has been reconstituted with the CODH complex, ferredoxin, and purified membranes which contain an hydrogenase linked to cytochrome *b* (50).

$$CO + H_2O \rightarrow CO_2 + H_2 \ (\Delta G^{\circ\prime} = -20 \text{ kJ/mol [6]}) \quad (7)$$

In addition, H_2 is a source of electrons for the reductive demethylation of CH_3-S-CoM in the soluble fraction of *M. thermophila* (23). Thus, it is reasonable to postulate that oxidation of the carbonyl group of acetate could be coupled to H_2 evolution by membrane-bound electron carriers and that H_2 is subsequently oxidized to provide electrons for the reduction of CoM-S-S-HTP. This hypothesis implies a potential for ATP synthesis coupled to H_2 evolution by a chemiosmotic mechanism; indeed, proton translocation is coupled to the oxidation of CO to CO_2 and H_2 in cell suspensions of acetate-grown *M. barkeri* (6). However, a membrane-bound electron transport chain without the participation of H_2 as an intermediate is equally possible.

Methane formation from acetate is dependent on sodium and is accompanied by the generation of a secondary sodium ion gradient (45); however, the sodium requirement is not understood. Two reactions that may be driven by a sodium ion potential are the uptake of acetate and the endergonic cleavage of acetyl-CoA (reaction 8).

$$CH_3CO\text{-S-CoA} + \text{HS-CoM} \rightarrow CO + CH_3\text{-S-CoM} + \text{CoA} \quad (\Delta G^{\circ\prime} = +40.3 \text{ kJ/mol [34]}) \quad (8)$$

OTHER ENZYME ACTIVITIES

The methyl group of acetate is primarily converted to methane; however, a small proportion of methyl groups is oxidized to CO_2 to provide electrons for reductive biosynthesis. Acetate-grown *Methanosarcina* species contain low levels of enzymes which are utilized in the CO_2-reduction pathway for methanogenesis; thus, it is proposed that the methyl group of acetate is oxidized by a reversal of the pathway for CO_2 reduction to methane (22). Acetate-grown *M. barkeri* contains formyl-methanofuran dehydrogenase and 5,10-methylene-H_4MPT (tetrahydromethanopterin) dehydrogenase (47) and acetate-grown *M. thermophila* contains formyl-methanofuran:H_4MPT formyltransferase, 5,10-methenyl-H_4MPT^+ cyclohydrolase, and F_{420}-dependent 5,10-methylene-H_4MPT dehydrogenase (22). The low levels of these enzymes suggest they are not involved in conversion of the methyl group of acetate to methane, but in oxidation of the methyl group of acetate to CO_2.

Growth of *M. barkeri* and *M. thermophila* on acetate induces carbonic anhydrase activity, but the function of this enzyme in the conversion of acetate to methane is unknown (22, 33). It is proposed that the formation of carbonic acid may be required in an antiport mechanism for transport of the acetate anion into the cell (33).

REGULATION OF ENZYME SYNTHESIS

The DNA-dependent RNA polymerases from members of the *Archaea* domain have a complex subunit composition

characteristic of the nuclear polymerases from members of the *Eucarya* domain and unlike the $\beta\beta'\alpha\alpha_2\sigma$ subunit composition of the polymerases from members of the *Bacteria* domain. These differences are reflected in the different consensus promoter sequences for the *Archaea* and *Bacteria* domains (20). Thus, the regulation of transcription in members of the *Archaea* domain is likely to involve molecular mechanisms which are fundamentally different from those the *Bacteria* domain.

Methanosarcina spp. are the most catabolically versatile of the methane-producing organisms and utilize, in addition to acetate, methanol and methylamines for growth and methanogenesis. Methanogenesis from methanol (reaction 9) yields more available energy than acetate conversion to methane.

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \quad (\Delta G^{\circ\prime} = -103 \text{ kJ/mol } CH_4 \text{ [55]}) \qquad (9)$$

Thus, cells grown with a mixture of these substrates utilize methanol first. Two-dimensional gel electrophoresis of cell extract proteins from *M. thermophila* reveal more than 100 mutually exclusive peptides in acetate- and methanol-grown cells (22). This regulation of enzyme synthesis in response to the growth substrate presents an opportunity for future investigations into the molecular basis for transcriptional regulation in members of the *Archaea* domain.

SUMMARY

The general features are known for the pathway by which most methane is produced in nature. All acetate-utilizing methanogenic microorganisms contain CODH which catalyzes the cleavage of acetyl-CoA; however, the pathway differs from all other acetate-utilizing anaerobes in that the methyl group is reduced to methane with electrons derived from oxidation of the carbonyl group of acetyl-CoA to CO_2. The current understanding of the methanogenic fermentation of acetate provides impressions of nature's novel solutions to problems of methyl transfer, electron transport, and energy conservation. The pathway is now at a level of understanding that will permit productive investigations of these and other interesting questions in the near future.

REFERENCES

1. **Abbanat, D. R., and J. G. Ferry.** 1990. Synthesis of acetyl-CoA by the carbon monoxide dehydrogenase complex from acetate-grown *Methanosarcina thermophila*. J. Bacteriol. **172:**7145–7150.
2. **Abbanat, D. R., and J. G. Ferry.** 1991. Resolution of component proteins in an enzyme complex from *Methanosarcina thermophila* catalyzing the synthesis or cleavage of acetyl-CoA. Proc. Natl. Acad. Sci. USA **88:**3272–3276.
3. **Aceti, D. J., and J. G. Ferry.** 1988. Purification and characterization of acetate kinase from acetate-grown *Methanosarcina thermophila*. J. Biol. Chem. **263:**15444–15448.
4. **Albracht, S. P. J., D. Ankel-Fuchs, R. Böcher, J. Ellermann, J. Moll, J. W. van der Zwaan, and R. K. Thauer.** 1988. Five new EPR signals assigned to nickel in methyl-coenzyme M reductase from *Methanobacterium thermoautotrophicum*, strain Marburg. Biochim. Biophys. Acta **941:**86–102.
5. **Aldrich, H. C., D. B. Beimborn, M. Bokranz, and P. Schönheit.** 1987. Immunocytochemical localization of methyl-coenzyme M reductase in *Methanobacterium thermoautotrophicum*. Arch. Microbiol. **147:**190–194.
6. **Bott, M., and R. K. Thauer.** 1989. Proton translocation coupled to the oxidation of carbon monoxide to CO_2 and H_2 in *Methanosarcina barkeri*. Eur. J. Biochem. **179:**469–472.
7. **Cao, X., and J. A. Krzycki.** 1991. Acetate-dependent methylation of two corrinoid proteins in extracts of *Methanosarcina barkeri*. J. Bacteriol. **173:**5439–5448.
8. **Clements, A. P., and J. G. Ferry.** 1992. Cloning, nucleotide sequence, and transcriptional analyses of the gene encoding a ferredoxin from *Methanosarcina thermophila*. J. Bacteriol. **174:**5244–5250.
9. **DiMarco, A. A., T. A. Bobik, and R. S. Wolfe.** 1990. Unusual coenzymes of methanogenesis. Annu. Rev. Biochem. **59:**355–394.
10. **Eggen, R. I. L., A. C. M. Geerling, A. B. P. Boshoven, and W. M. de Vos.** 1991. Cloning, sequence analysis, and functional expression of the acetyl coenzyme A synthetase gene from *Methanothrix soehngenii* in *Escherichia coli*. J. Bacteriol. **173:**6383–6389.
11. **Eggen, R. I. L., A. C. M. Geerling, M. S. M. Jetten, and W. M. de Vos.** 1991. Cloning, expression, and sequence analysis of the genes for carbon monoxide dehydrogenase of *Methanothrix soehngenii*. J. Biol. Chem. **266:**6883–6887.
12. **Fischer, R., and R. K. Thauer.** 1988. Methane formation from acetyl phosphate in cell extracts of *Methanosarcina barkeri*. Dependence of the reaction on coenzyme A. FEBS Lett. **228:**249–253.
13. **Fischer, R., and R. K. Thauer.** 1989. Methyltetrahydromethanopterin as an intermediate in methanogenesis from acetate in *Methanosarcina barkeri*. Arch. Microbiol. **151:**459–465.
14. **Fischer, R., and R. K. Thauer.** 1990. Ferredoxin-dependent methane formation from acetate in cell extracts of *Methanosarcina barkeri* (strain MS). FEBS Lett. **269:**368–372.
15. **Grahame, D. A.** 1989. Different isozymes of methylcobalamin-2-mercaptoethanesulfonate methyltransferase predominate in methanol-grown versus acetate-grown *Methanosarcina barkeri*. J. Biol. Chem. **264:**12890–12894.
16. **Grahame, D. A.** 1991. Catalysis of acetyl-CoA cleavage and tetrahydrosarcinapterin methylation by a carbon monoxide dehydrogenase-corrinoid enzyme complex. J. Biol. Chem. **266:**22227–22233.
17. **Grahame, D. A., and T. C. Stadtman.** 1987. *In vitro* methane and methyl coenzyme M formation from acetate: evidence that acetyl-CoA is the required intermediate activated form of acetate. Biochem. Biophys. Res. Commun. **147:**254–258.
18. **Grahame, D. A., and T. C. Stadtman.** 1987. Carbon monoxide dehydrogenase from *Methanosarcina barkeri*. Disaggregation, purification, and physiochemical properties of the enzyme. J. Biol. Chem. **262:**3706–3712.
19. **Harder, S. R., W.-P. Lu, B. A. Feinberg, and S. W. Ragsdale.** 1989. Spectroelectrochemical studies of the corrinoid iron-sulfur protein involved in acetyl coenzyme-A synthesis by *Clostridium thermoaceticum*. Biochemistry **28:**9080–9087.
20. **Hausner, W., G. Frey, and M. Thomm.** 1991. Control regions of an archaeal gene. A TATA box and an initiator element promote cell-free transcription of the $tRNA^{val}$ gene of *Methanococcus vannielii*. J. Mol. Biol. **222:**495–508.
21. **Hedderich, R., A. Berkessel, and R. K. Thauer.** 1989. Catalytic properties of the heterodisulfide reductase involved in the final step of methanogenesis. FEBS Lett. **255:**67–71.
22. **Jablonski, P. E., A. A. DiMarco, T. A. Bobik, M. C. Cabell, and J. G. Ferry.** 1990. Protein content and enzyme activities in methanol- and acetate-grown *Methanosarcina thermophila*. J. Bacteriol. **172:**1271–1275.
23. **Jablonski, P. E., and J. G. Ferry.** 1991. Purification and properties of methyl coenzyme M methylreductase from acetate-grown *Methanosarcina thermophila*. J. Bacteriol. **173:**2481–2487.
24. **Jablonski, P. E., W.-P. Lu, S. W. Ragsdale, and J. G. Ferry.** 1991. EPR spectroelectrochemical studies of the carbon monoxide dehydrogenase enzyme complex, nickel/iron-sulfur protein, and corrinoid/iron-sulfur protein of acetate-grown *Methanosarcina thermophila*, abstr. K-136, p. 237. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991. American Society for Microbiology, Washington, D.C.
25. **Jaun, B., and A. Pfaltz.** 1988. Coenzyme F_{430} from methanogenic bacteria: methane formation by reductive carbon-sulfur bond cleavage of methyl sulphonium ions catalysed by F_{430}

pentamethyl ester. J. Chem. Soc. Chem. Commun. **1988**:293–294.
26. **Jetten, M. S. M., W. R. Hagen, A. J. Pierik, A. J. M. Stams, and A. J. B. Zehnder.** 1991. Paramagnetic centers and acetyl-coenzyme A/CO exchange activity of carbon monoxide dehydrogenase from *Methanothrix soehngenii*. Eur. J. Biochem. **195**:385–391.
27. **Jetten, M. S. M., A. J. Pierik, and W. R. Hagen.** 1991. EPR characterization of a high-spin system in carbon monoxide dehydrogenase from *Methanothrix soehngenii*. Eur. J. Biochem. **202**:1291–1297.
28. **Jetten, M. S. M., A. J. M. Stams, and A. J. B. Zehnder.** 1989. Isolation and characterization of acetyl-coenzyme A synthetase from *Methanothrix soehngenii*. J. Bacteriol. **171**:5430–5435.
29. **Jetten, M. S. M., A. J. M. Stams, and A. J. B. Zehnder.** 1989. Purification and characterization of an oxygen-stable carbon monoxide dehydrogenase of *Methanothrix soehngenii*. FEBS Lett. **181**:437–441.
30. **Jetten, M. S. M., A. J. M. Stams, and A. J. B. Zehnder.** 1990. Acetate threshold values and acetate activating enzymes in methanogenic bacteria. FEMS Microbiol. Ecol. **73**:339–344.
31. **Jetten, M. S. M., A. J. M. Stams, and A. J. B. Zehnder.** 1990. Purification and some properties of the methyl-CoM reductase of *Methanothrix soehngenii*. FEMS Microbiol. Lett. **66**:183–186.
32. **Jones, W. J., D. P. Nagle, and W. B. Whitman.** 1987. Methanogens and the diversity of archaebacteria. Microbiol. Rev. **51**: 135–177.
33. **Karrasch, M., M. Bott, and R. K. Thauer.** 1989. Carbonic anhydrase activity in acetate grown *Methanosarcina barkeri*. Arch. Microbiol. **151**:137–142.
34. **Keltjens, J. T., and C. van der Drift.** 1986. Electron transfer reactions in methanogens. FEMS Microbiol. Rev. **39**:259–303.
35. **Kemner, J. M., J. A. Krzycki, R. C. Prince, and J. G. Zeikus.** 1987. Spectroscopic and enzymatic evidence for membrane-bound electron transport carriers and hydrogenase and their relation to cytochrome b function in *Methanosarcina barkeri*. FEMS Microbiol. Lett. **48**:267–272.
36. **Krzycki, J. A., L. E. Mortenson, and R. C. Prince.** 1989. Paramagnetic centers of carbon monoxide dehydrogenase from aceticlastic *Methanosarcina barkeri*. J. Biol. Chem. **264**:7217–7221.
37. **Krzycki, J. A., and R. C. Prince.** 1990. EPR observation of carbon monoxide dehydrogenase, methylreductase and corrinoid in intact *Methanosarcina barkeri* during methanogenesis from acetate. Biochim. Biophys. Acta **1015**:53–60.
38. **Ladapo, J., and W. B. Whitman.** 1990. Method for isolation of auxotrophs in the methanogenic archaebacteria: role of the acetyl-CoA pathway of autotrophic CO_2 fixation in *Methanococcus maripaludis*. Proc. Natl. Acad. Sci. USA **87**:5598–5602.
39. **Lin, S. K., and B. Jaun.** 1991. Coenzyme F_{430} from methanogenic bacteria: detection of a paramagnetic methylnickel (II) derivative of the pentamethyl ester by ^{2}H-NMR spectroscopy. Helv. Chim. Acta **74**:1725–1738.
40. **Lindahl, P. A., E. Münck, and S. W. Ragsdale.** 1990. CO dehydrogenase from *Clostridium thermoaceticum*. EPR and electrochemical studies in CO_2 and argon atmospheres. J. Biol. Chem. **265**:3873–3879.
41. **Lindahl, P. A., S. W. Ragsdale, and E. Münck.** 1990. Mössbauer study of CO dehydrogenase from *Clostridium thermoaceticum*. J. Biol. Chem. **265**:3880–3888.
42. **Lu, W.-P., S. R. Harder, and S. W. Ragsdale.** 1990. Controlled potential enzymology of methyl transfer reactions involved in acetyl-CoA synthesis by CO dehydrogenase and the corrinoid/iron-sulfur protein from *Clostridium thermoaceticum*. J. Biol. Chem. **265**:3124–3133.
43. **Lu, W.-P., and S. W. Ragsdale.** 1991. Reductive activation of the coenzyme A/acetyl-CoA isotopic exchange reaction catalyzed by carbon monoxide dehydrogenase from *Clostridium thermoaceticum* and its inhibition by nitrous oxide and carbon monoxide. J. Biol. Chem. **266**:3554–3564.
44. **Lundie, L. L., and J. G. Ferry.** 1989. Activation of acetate by *Methanosarcina thermophila*. Purification and characterization of phosphotransacetylase. J. Biol. Chem. **264**:18392–18396.
45. **Peinemann, S., V. Müller, M. Blaut, and G. Gottschalk.** 1988. Bioenergetics of methanogenesis from acetate by *Methanosarcina barkeri*. J. Bacteriol. **170**:1369–1372.
46. **Raybuck, S. A., S. E. Ramer, D. R. Abbanat, J. W. Peters, W. H. Orme-Johnson, J. G. Ferry, and C. T. Walsh.** 1991. Demonstration of carbon-carbon bond cleavage of acetyl coenzyme A by using isotopic exchange catalyzed by the CO dehydrogenase complex from acetate-grown *Methanosarcina thermophila*. J. Bacteriol. **173**:929–932.
47. **Schwörer, B., and R. K. Thauer.** 1991. Activities of formylmethanofuran dehydrogenase, methylenetetrahydromethanopterin dehydrogenase, methylenetetrahydromethanopterin reductase, and heterodisulfide reductase in methanogenic bacteria. Arch. Microbiol. **155**:459–465.
48. **Terlesky, K. C., M. J. Barber, D. J. Aceti, and J. G. Ferry.** 1987. EPR properties of the Ni-Fe-C center in an enzyme complex with carbon monoxide dehydrogenase activity from acetate-grown *Methanosarcina thermophila*. Evidence that acetyl-CoA is a physiological substrate. J. Biol. Chem. **262**:15392–15395.
49. **Terlesky, K. C., and J. G. Ferry.** 1988. Purification and characterization of a ferredoxin from acetate-grown *Methanosarcina thermophila*. J. Biol. Chem. **263**:4080–4082.
50. **Terlesky, K. C., and J. G. Ferry.** 1988. Ferredoxin requirement for electron transport from the carbon monoxide dehydrogenase complex to a membrane-bound hydrogenase in acetate-grown *Methanosarcina thermophila*. J. Biol. Chem. **263**:4075–4079.
51. **Thauer, R. K.** 1990. Energy metabolism of methanogenic bacteria. Biochim. Biophys. Acta **1018**:256–259.
52. **Thauer, R. K., D. Moller-Zinkhan, and A. M. Spormann.** 1989. Biochemistry of acetate catabolism in anaerobic chemotrophic bacteria. Annu. Rev. Microbiol. **43**:43–67.
53. **Thomas, I., H.-C. Dubourguier, G. Presier, P. Debeire, and G. Albagnac.** 1987. Purification of component C from *Methanosarcina mazei* and immunolocalization in *Methanosarcinaceae*. Arch. Microbiol. **148**:193–201.
54. **van de Wijngaard, W. M. H., C. van der Drift, and G. D. Vogels.** 1988. Involvement of a corrinoid enzyme in methanogenesis from acetate in *Methanosarcina barkeri*. FEMS Microbiol. Lett. **52**:165–172.
55. **Vogels, G. D., J. T. Keltjens, and C. van der Drift.** 1988. Biochemistry of methane production, p. 707–770. *In* A. J. B. Zehnder (ed.), Biology of anaerobic microorganisms. Wiley, New York.
56. **Whitman, W. B.** 1985. Methanogenic bacteria, p. 3–84. *In* I. C. Gunsalus, J. R. Sokatch, and L. N. Ornston (ed.), The bacteria, a treatise on structure and function. Academic Press, Orlando, Fla.
57. **Woese, C. R., O. Kandler, and M. L. Wheelis.** 1990. Towards a natural system of organisms. Proposal for the domains archaea, bacteria, and eucarya. Proc. Natl. Acad. Sci. USA **87**:4576–4579.